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Particle Capture by the Gills of *Dreissena polymorpha:* Structure and Function of Latero-frontal Cirri

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Abstract. Microscopic techniques were used to examine the role of gill cirri in particle capture by *Dreissena polymorpha.* The latero-frontal cirri, formed from two fused ciliary plates, consist of about 40 pairs of cilia. Each cilium in the plate contains a typical 9 + 2 axoneme in the fused region of the cirrus, but the structure of the axoneme in the long, free ciliary tips is reduced. The cilia in a cirrus are graded in length, with the shortest cilia positioned frontally. The cirral cilia move in unison, allowing the cirrus to move from a flexed position with its tip arched over the front of the gill filament, to an extended position with the cirrus projected in the plane of the latero-frontal cell and extending across the interfilament space. In the latter position, the free ciliary tips of opposing and neighboring cirri form a "trap" (net) with a spacing of about 0.5 μm. Observations with laser confocal microscopy indicated that these structures can physically trap particles ≤ 1 μm in diameter. Particles captured by the extended cirri are moved to the frontal surface of the gill, where the cirri are swept by the lateralmost frontal cilia. During cirral movement the shift from extended to flexed position is, in part, achieved by the base of the cirrus pivoting at a hinge region. Morphologically, the hinge region shows axonemal specializations that consist of electron-dense plates and other structures of undefined function that may be important in the overall movement of the cirrus. In addition to trapping by cirri, we also observed particles moving in the water currents, particularly in the frontal current located over the apical surface of the filament, suggesting that some particles are captured in these water currents without being physically trapped. Probably, therefore, trapping by the cirri and establishment of water currents by the filaments both participate in the interception of particles by *Dreissena polymorpha.*

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Abbreviations: CC—cirral cilia; PW—pondwater; SEM—scanning electron microscopy; TEM—transmission electron microscopy.

Introduction

Understanding of suspension feeding in lamellibranch bivalves requires a knowledge of the factors responsible for water movement through the mantle cavity and across the gills. In addition to water flow characteristics, the mechanisms of particle capture, transport, selection, and ingestion in a particular species define the abilities and the limitations of suspension feeding in that species. The organ primarily responsible for particle capture is the gill. Morton (1983) reviewed this topic, and describes the filtration (suspension feeding) mechanism to be "largely a function of eulaterofrontal cilia, although mucus and patterns of water flow over the surface of the ctenidia may play an additional role in filter feeding." The generalized role of water flow patterns in suspension feeding has been reviewed by Jørgensen (1990), and the role played by mucus in particle manipulation has been elucidated by Beninger and colleagues for various marine species (Beninger *et al.,* 1991, 1992, 1993; Ward *et al.,* 1993), and by Tankersley and Dimock (1993) for a freshwater unionid.

In eulamellibranch gills, particles are captured from water that is driven across the gill filaments by the motion of the lateral cilia on each side of the filaments (Gibbons, 1961; Jørgensen, 1982, 1989). Latero-frontal cells and their complex motile organelles, either cilia or cirri composed of many fused cilia, are located between the frontal surface of the filament and the lateral ciliated cells. Atkins (1938) describes the cirri in a variety of bivalves and notes their widespread occurrence among lamellibranchs. Variation in the latero-frontal ciliary appendages of different bivalves ranges from single cilia to

those formed by two fused ciliary plates containing as many as 40 cilia per plate (Atkins, 1938). The cilia forming the fused cirral plates have free ciliary tips that form what appear to be nets or sieves in the interfilament space between gill filaments (Atkins, 1937; Moore, 1971; Owen and McCrae, 1976; Ribelin and Collier, 1977).

Cirral ultrastructure and the structure of the interfilament sieve has been described for many marine species (Dral, 1967; Gibbons, 1961; Moore, 1971; Owen, 1974; Owen and McCrae, 1976; Ribelin and Collier, 1977), and the beat pattern of an individual cirrus on the gill of *Mytilus edulis* has been described by Dral (1966, 1967). Several attempts to correlate the efficiency of particle capture with species differences in cirral structure have led to conflicting interpretations and conclusions.

If one assumes that 1 μm particles should be retained at 100% efficiency by cirral nets, then the experimental results of some feeding experiments do not support a cirral particle capture mechanism (Vahl, 1973; Møhlenberg and Riisgård, 1978; Riisgård, 1988). Others have concluded that the latero-frontal cirri participate in mechanical filtration (Atkins, 1938; Dral, 1967; Moore, 1971; Owen and McCrae, 1976; Silvester and Sleigh, 1984). But the lack of cirri in some bivalve families, as well as hydrodynamic considerations, led Nielsen *et al.* (1993) to suggest that cirri may be specialized structures that improve the retention efficiency of the gill for small particles. Small particle retention efficiency varies among species with cirri of different sizes (Vahl, 1973; Møhlenberg and Riisgård, 1978; Kryger and Riisgård, 1988; Silverman *et al.*, 1995), with those species having complex cirri showing the highest efficiency. To date, the functions of cirri in particle capture have been attributed to their role in complex current formation, their contribution to overall gill hydrodynamics, or to mechanical filtration. These roles are not mutually exclusive, and perhaps a combination of mechanisms allows bivalves with complex cirri to retain smaller particles.

Dreissena polymorpha is a eulamellibranch filter feeder capable of removing particulates <1 μm in diameter (Sprung and Rose, 1988). Particles filtered include single-celled algae, diatoms, and some bacteria (Mikheev and Sorokin, 1966; Morton, 1971; Ten Winkel and Davids, 1982; Jørgensen *et al.*, 1984; Silverman *et al.*, 1995). In this paper, we use laser confocal microscopy to describe cirri and their movements in *D. polymorpha*. The gill cirri of *D. polymorpha* are complex as described by Atkins (1938). An advantage of laser confocal microscopy is its selectable image plane and depth of field coupled with a high resolution (approaching 0.2 μm). The results indicate that, in isolated gill preparations, cirri participate in the capture of small particles.

Materials and Methods

Preparation and fixation of gill tissue for microscopy

Dreissena polymorpha were collected from Lake Erie and from the Mississippi River near Baton Rouge, Louisiana. Animals were maintained in the laboratory at 4–15°C in pondwater and were acclimated to 23°C for 3–5 days before use (Dietz *et al.*, 1994). During this study, the gills from more than fifty animals were examined microscopically.

The standard tissue fixation was accomplished by inserting a syringe needle into the byssal opening in the shell, injecting phosphate-buffered 2% glutaraldehyde (pH 7.2) into the intact animal, and then immersing the entire animal in fixative for 1 h. The concentration of the phosphate buffer was adjusted to match the measured osmolality of *D. polymorpha* blood, about 35–45 mosm. The gills of all freshwater bivalves are extremely sensitive to the osmolality of the fixative. For *D. polymorpha*, an osmotic imbalance of 10 mosm in either direction of isosmolarity resulted in an unsuitable preparation (see Silverman *et al.*, 1996). Following the 1-h immersion in fixative, the animal was dissected and 1–6 mm gill strips were placed in fresh, buffered glutaraldehyde for an additional hour. Alternatively, some gills were carefully dissected without pre-fixation and pinned in a desired orientation before a 1-h glutaraldehyde fixation. All tissues were rinsed in isosmotic phosphate buffer and post-fixed for 1 h in 1% osmium tetroxide. Tissues for light and transmission electron microscopy (TEM) were dehydrated in a graded alcohol series and embedded in LR White (London Resin Co., Reading, England) methacrylate resin cured at 60°C (Gardiner *et al.*, 1991a).

Gills were sectioned with a Reichert-Jung Ultracut E ultramicrotome at a thickness of 0.5–2.0 μm for light microscopy and at 60–90 nm for electron microscopy. Sections for light microscopy were stained with borate buffered 1% toluidine blue containing 0.5% borax and then examined with a Nikon Microphot-FXA microscope equipped with Planapochromat lenses. Sections for TEM were stained with 2% uranyl acetate for 10 min followed by lead citrate for 1 min (Reynolds, 1963). Thin sections were examined with a JEOL 100CX transmission electron microscope operating at 80 kV.

For observation with the scanning electron microscope (SEM), some gills were pre-treated with 10^{-6} *M* serotonin. As in unionid gills (Gardiner *et al.*, 1991a), 10^{-6} *M* serotonin increases cirral beat and relaxes the branchial musculature in *D. polymorpha*, thus increasing ostial diameter and interfilament distance (Gardiner *et al.*, 1991a, b). This effect facilitated the observation of the motile organelles on the filament surface. For SEM, fixation was as described above with all specimens dehydrated in a graded alcohol series. Specimens were

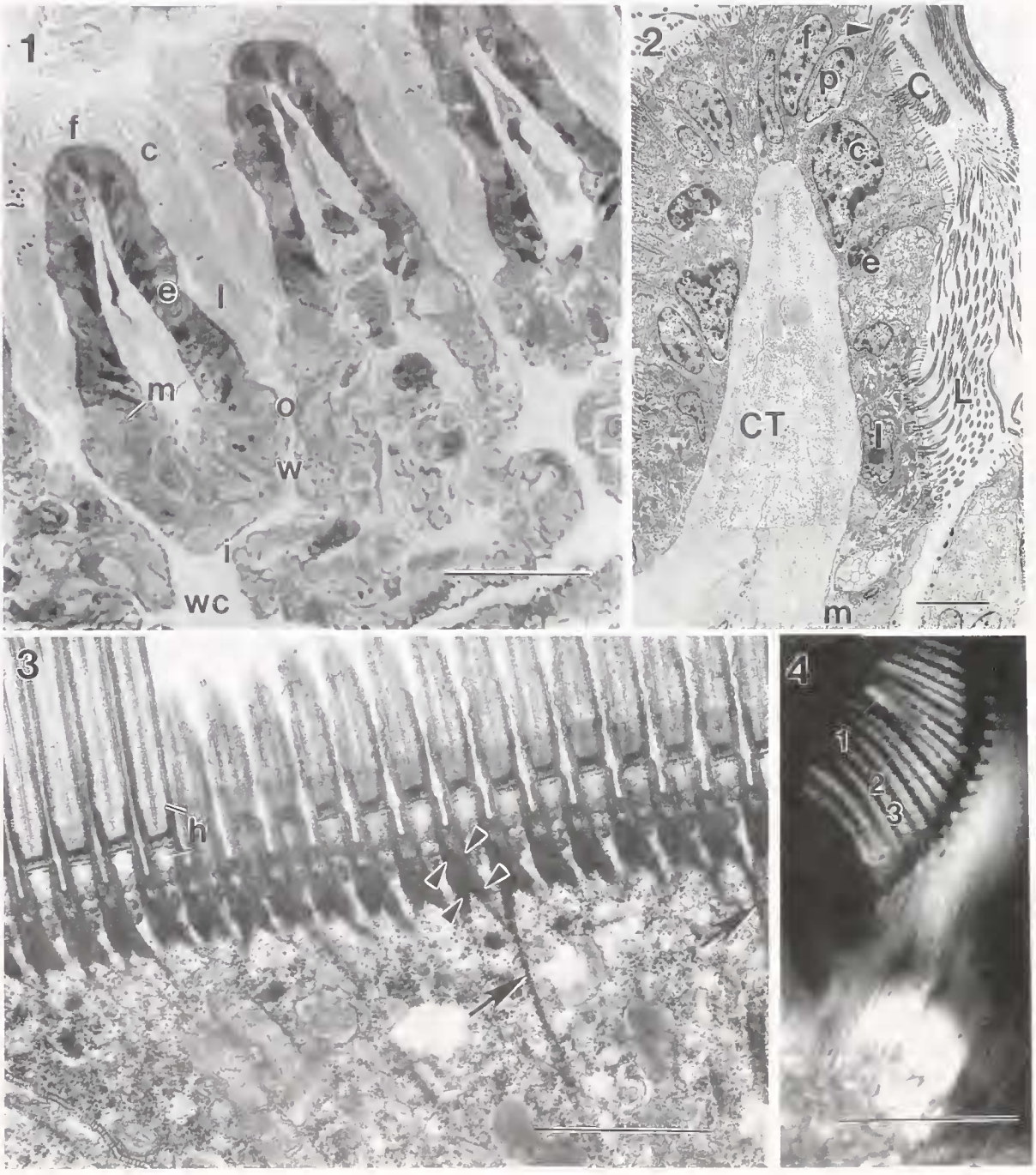


Figure 1. A section through the gill of *Dreissena polymorpha* showing portions of three filaments stained with toluidine blue. Note the relative position of frontal cilia (f), latero-frontal cirri (c), and lateral cilia (l), along the filament. In addition to the three cell types with cilia, there is a set of indifferent epithelial cells (e) between the lateral ciliated cells and the latero-frontal cells. Water moves from the surface of the filaments through the ostial opening (o) into the water canal (w), through the internal ostia (i) and into the central water channel (wc). The water canals on both sides of a filament enter the central water channel through one common internal opening. Note that there are no goblet-type mucus (m)-producing cells among the cells making up the surface epithelium of the filament, but they are present at the filament base near the ostial opening and in the central water channel. Bar = 25 μm.

Figure 2. An electron micrograph collage showing the epithelial cells of the filament: frontal epithelial cells (f); pro-lateral-frontal epithelial cells (p), latero-frontal cirral cells (c); indifferent epithelial cell (e); lateral ciliated cell (l); mucus cell (m), cirri (C), pro-latero-frontal cilia (arrowhead), and lateral cilia (L). The connective tissue skeleton of the filament (CT) is also notable. Bar = 5 μm.

wrapped in lens paper to prevent curling, critical point dried (Denton Vacuum, Inc), and mounted on stubs with double-sided carbon tape. Specimens were sputter-coated with gold and palladium (20 nm) and viewed with a Cambridge 2000 scanning electron microscope operating at 10–15 kV.

Gill visualization by laser confocal microscopy

Because gill tissue displays some autofluorescence and reflectance, observations of the gill by laser confocal microscopy can be made either with or without fluorescent markers. For specific enhancement of cilia and cirri, FITC-colchicine (FITC-C; Molecular Probes, Inc.) was used. Gills were carefully removed from an animal and placed in freshwater clam Ringer's solution (Gardiner *et al.*, 1991b). Some of these gills, unfixed, were split along the septa into two lamellae by microdissection. The cilia and musculature of gills prepared in this way remained active for more than 12 h. Gill strips to be fluorescently labelled were separated and placed in 20 μg/ml FITC-C for 10–30 min. During exposure to fluorescent dyes, the gills were protected from light. The gill strip was removed from the marker dye, washed in several changes of Ringer's solution, and maintained in the dark until used.

Gills were oriented and mounted on a slide under a dissecting microscope. Some gills were mounted on open mesh Nitex screen (125 μm mesh), which allowed water to flow more readily across the gill. A coverslip supported at the edges by silicone vacuum grease was suspended over the preparation. This arrangement was necessary to keep the coverslip from interfering with ciliary and cirral motion. The specimen was examined with a Noran Instruments, Inc., laser confocal system mounted on a Nikon Optiphot microscope equipped with fluorescence objectives. The excitation wavelength was 529 nm from an argon ion laser for all preparations, and an FITC barrier filter was used. The video image consisted of both a reflective and a fluorescent component. Images were captured and analyzed with Odyssey InterVision (Noran Instruments, Inc.) hardware and software on a Silicon Graphics Indy computer.

In other experiments, fluorescent beads (FITC) of 1.0 μm and 0.75 μm diameter were used to track water flow and particle handling by the gill. The FITC-beads were added to the slide at the dorsal end of the gill. This allowed particle movement to be tracked across the gill, and particle interaction with the surface components of the gill to be visualized. In these experiments, no FITC-colchicine was used, and we relied on autofluorescence and reflectance to visualize the cirri.

Measurement of ciliary beat frequency

Beat frequencies of the cirri and lateral cilia were determined with an Intervision software program that records changes in brightness over time. With the confocal microscope focused at the appropriate ciliary group, a small function box was superimposed on the image such that a single cirrus, cilium, or a group of lateral cilia passed though the box with each beat. Because cilia are imaged as bright structures on a darker background, a graph of continuous beating could be captured over any time interval and displayed as peaks in the digital "strip chart" plotted against time. Over a given period, the peaks were counted for three areas in five excised non-stimulated gills and the values averaged to give the beat frequency for the cirri and lateral cilia. Because of variability between and even within gills, we have reported these values as range estimates rather than as statistical averages. The isolated gill preparations used here are differentially sensitive to neurohormonal agents and their pharmacologic analogs (unpubl. obs.; Gardiner *et al.*, 1991a, b). We have not reported the effects of neurohormonal agents in this study. We frequently used serotonin to stimulate quiescent cilia to verify tissue viability and responsiveness.

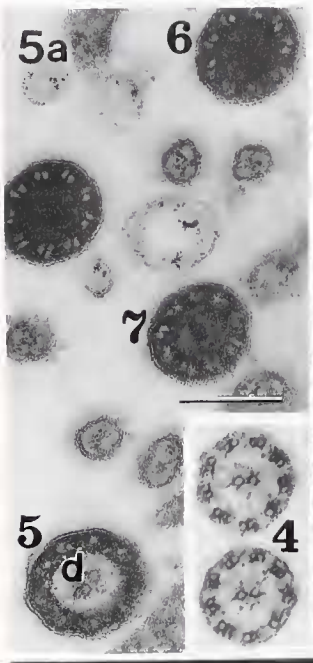
Results

General structure of the gill filament of D. polymorpha

Morton (1993) has described the structure of the *D. polymorpha* gill as conforming to the general eulamellibranch pattern (Fig. 1). Most descriptions of water flow

Figure 3. Transmission electron micrograph of the apical portion of a latero-frontal cell showing the basal portion of a single cirrus composed of numerous cilia. The extensive system of rootlets (arrows) underlying the cirrus is evident. Note that these rootlets are anchored in electron-dense structures that envelop the microtubules at the base of each cilium (arrowheads). These dense structures link the microtubules on the right side of one cilium to the left side of the next cilium. The hinge or neck region (h) of each cilium is also visible. Bar = 1 μm.

Figure 4. Single Z-plane image of a cirrus, one of a series of images captured every 33 ms by laser confocal microscopy. This cirrus was moving toward the flexed position where it would partially cover the frontal cilia. The fused plate component of the cirrus consisting of individual cilia is located at the bottom of the micrograph. The free tips of the individual cilia are at the top of the micrograph. Positions **1**, **2**, and **3** on the free tips show reduced microtubule numbers from the normal "9 + 2" axoneme structure and correspond to **1**, **2**, and **3** in Figure 5c. Bar = 5 μm.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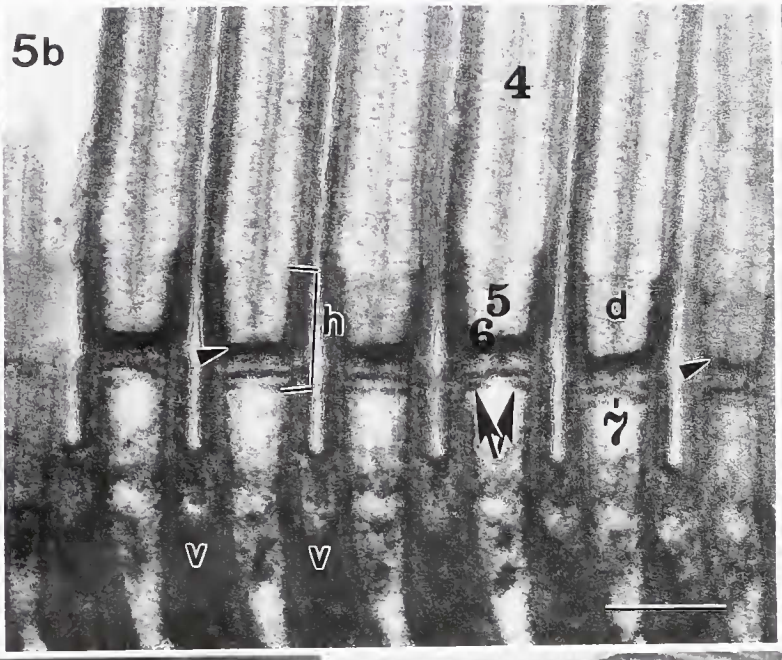
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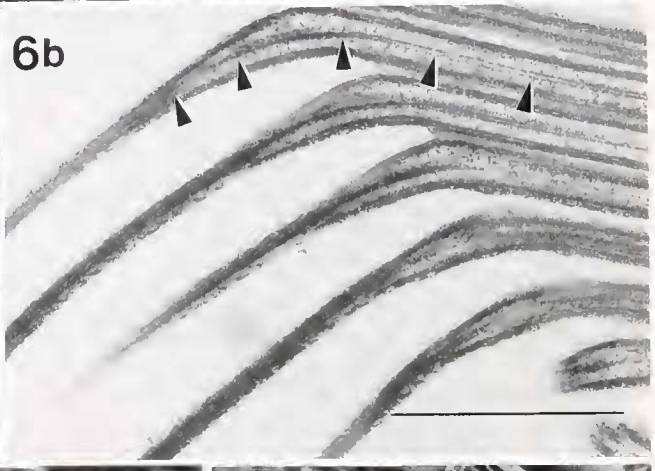
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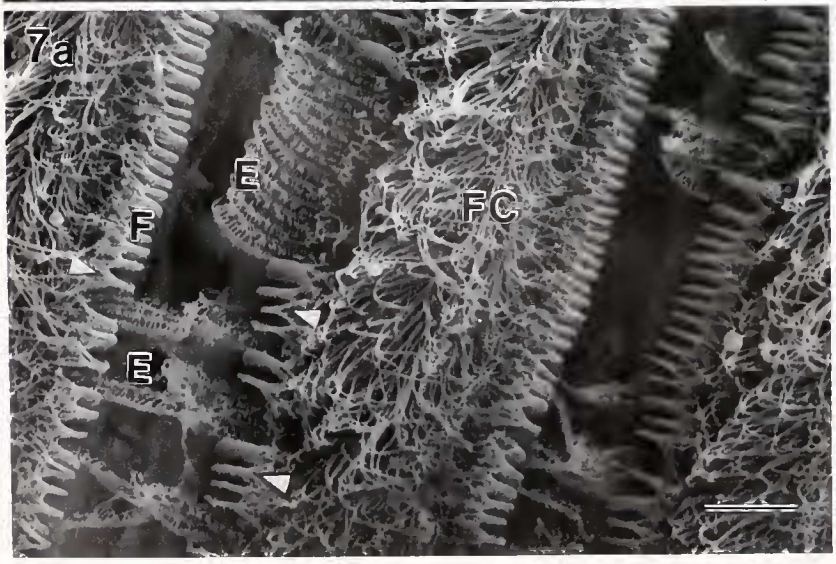
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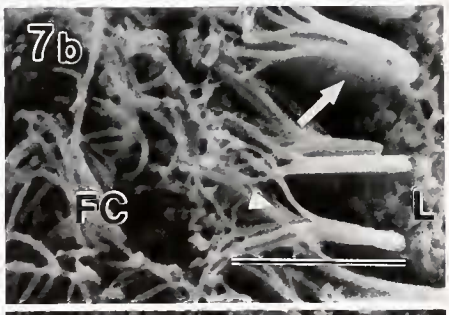
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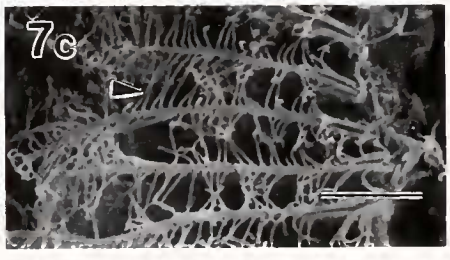
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across such gills indicate that the lateral ciliated cells create the force for water flow through the interfilament ostia (Gibbons, 1961; Jørgensen, 1981, 1982, 1989). A detailed examination of the filament epithelium of *D. polymorpha* revealed that all cells contain microvilli, and that there are at least five different cell types (Figs. 1, 2). Three of these cell types have motile appendages: the frontal and the lateral ciliated cells contain cilia as individual structures, but the latero-frontal cells contain cirri composed of fused cilia. The frontal cells closest to the latero-frontal cells have longer cilia than the rest of the frontal cells, and these correspond to the pro-lateral-frontal cells described in many species with large latero-frontal cirri (Atkins, 1938). There were 3–4 lateral ciliated cells and a latero-frontal cell in a cross section through the filament (Fig. 1). A slightly oblique cross section may show multiple latero-frontal cells. Between the lateral ciliated cells and the latero-frontal cells is a nonciliated cell (Fig. 2). Between the lateral ciliated cells and the base of a filament (near the ostial opening) are other nonciliated cells (Fig. 1). The only goblet-shaped mucus cells seen were located at the base of the filament surrounding the external ostia leading to the central water channel of the gill (Figs. 1, 2). The water channel epithelium also contains many mucus cells, especially around the internal ostia and on the septa (not shown).

Laser confocal microscopy allowed observation of the characteristic beating patterns associated with each of the three ciliary appendage types. The frontal cilia on the outer surface of the filament propel material ventrally toward the food groove on the gill margin. Each frontal cilium displays a typical ciliary beat consisting of a power stroke and recovery stroke (Sleigh, 1962). Observation of the lateral ciliated cells confirmed a powerful rhythmic,

Figure 5. High magnification transmission electron micrographs through various regions of a cirrus. Cross sections through various regions of the cilium are identified by number (**1–7**) increasing from tip to base. (a) Cross-sectional images through cirral components; individual cilia from a cirrus have separated during fixation, but cross-sectional images at different levels allow a comparison with the longitudinal images. The inset shows the normal 9 + 2 axonemal pattern seen throughout the fused portion of the cirrus. (b) A high magnification longitudinal micrograph through the hinge region of Figure 3. (c) Cross section of a cirrus made in the region of the ciliary tips. Note in the longitudinal section that the hinge region [h] is narrower than the rest of the axoneme (Figs. 3, 5b). The normal axoneme and its corresponding cross section (in Fig. 5a, b) are indicated by **4**. The hinge region is 0.2 μm long; for most of this length, the axonemal microtubules are surrounded by an electron-dense material (**5** in Fig. 5a, b). The central microtubular doublets are present in this region (d). Toward the base of the cilia, the hinge region appears to be continuous with an electron-dense plate (arrowhead) such that the electron-dense material actually forms a cup on the basal end of the cilium proper. The outer doublets, but not the central pair of microtubules, appear to penetrate this plate (**6** in Figs. 5a, b). Below this plate is a second thinner electron-dense plate (**7** in Figs. 5a, b). Finally, a cone of additional electron-dense material (arrows) connects the outer microtubular doublets to the center of the thick electron-dense plate immediately below the level where the central doublets end. The connections of outer microtubular doublets at the base of the cilia described in Figure 3 are also visible (v) connecting adjacent cilia. The larger cross sections in 5c contain both microtubular doublets and singlets. These ciliary cross sections are distal to the shoulder region and the number of singlets is less than the original nine microtubular doublets of the axoneme. Note the eccentric position of the central doublet (arrowhead) and its position with reference to the remaining outer doublet (arrow). Progressing toward the ciliary tip the diameter and number of microtubules in the axoneme decreases. The relative positions at which these axonemal structures are present is identified as **1**, **2**, and **3** in Figure 4. Bars = 0.2 μm.

Figure 6. Electron micrographs of the shoulder region of a cirrus where the free tips of the cilia emerge from the fused body region. (a) The scanning electron micrograph shows the abrupt angle formed between the free ciliary tips and the fused body of the cirrus. (b) A comparable transmission electron micrograph of this same region indicates that, at the shoulder region, the central doublet (arrowheads) does not follow the angle taken by the tip but instead becomes closely associated with the outer doublet on the edge opposite the one forming the internal angle. The association is with either outer doublet 1 or 5 depending on the cirral sheet to which the cilia belong. Bars: a = 2.5 μm; b = 1 μm.

Figure 7. Scanning electron micrographs of *Dreissena polymorpha* showing (a) portions of three gill filaments. In this low magnification image cirri are in both the flexed (F) and extended (E) positions. In the flexed position cilia from the two cirral plates form the shape of a "V" (white arrowheads) as they bend over the frontal cilia (FC) of the filament. (b) Higher magnification of the cirri in the flexed position. Note the fused body of an individual cirrus (arrow) and the "V" shape formed by the free tips (white arrowhead). The "V"-like form (white arrowhead) is observed as the free tips move over the frontal cilia (FC). The tips of the lateral cilia (L) are also visible. (c) Higher magnification with cirri in the extended position showing that the free ciliary tips extend between adjacent cirral plates forming a net-like structure. The black arrowhead indicates one such tip. The size of the spaces in the net ranges from 0.2 to 0.7 μm, with most being in the 0.4–0.5 μm range. Bars: a = 10 μm; b = 5 μm; c = 5 μm.

metachronal beat composed of waves of ciliary movement (about 4/s) that draw water down between the filaments and into the ostia. The lateral cilia beat in the axis of the filament. The attendant water flow appeared to be organized primarily along the length of the filament, but the location of the underlying ostia and their influence on the flow was evident. A somewhat circular current pattern over the underlying ostia was revealed by the motion of suspended fluorescent particles (data not shown). Also associated with the metachronal beat of the lateral cilia was a rhythmic movement of adjacent gill filaments. We could not determine whether the cilia were causing the filaments to move, or whether the filaments were moving rhythmically due to muscle contraction in synchrony with the ciliary beating.

Spontaneous cirral beating was more irregular than that of the lateral or frontal cilia in the isolated gills. Most often the cirri cycled rapidly and synchronously; at other times some cirri were cycling, and a few were acting independently or were motionless. The beat of the cirri was usually slower than that of the lateral ciliary beat, but we observed beat frequencies ranging from 3 to 15 beats/s in most gills. The wave form of the latero-frontal cirri was very different from that of either the frontal cilia or the lateral cilia and was based on the unique structure of these organelles (described below).

Cirral structure

An anterior-posterior cross section of the gill demonstrates the location of the cirri on the gill filaments (Fig. 1). Cirri originate from the apical end of latero-frontal cells and extend into the interfilament space (Figs. 1–3). A single latero-frontal cirrus is composed of a pair of fused ciliary sheets (or plates) both of which exit a single latero-frontal cell. A single cirral plate is shown in Figure 4. The ciliary basal bodies at the base of each cirral plate are arranged linearly, 38–42 in a row, with two parallel rows in each cell forming the base of a cirrus. Each row of basal bodies is aligned parallel to the anterior-posterior plane of Figure 1. The basal bodies of adjacent cilia in a cirral plate are tied together by electron-dense structures (Fig. 3) that connect an individual cilium with its neighbor. Each cirral cilium is connected through the electron-dense structures (Figs. 3, 5) to long intracellular rootlets that extend to the base of the cell (Fig. 3). Rootlet anchoring of cirral cilia is not uncommon for other types of cirri found in a variety of organisms (Gibbons, 1961; Sleigh, 1962). No other connections distal to the basal body between the individual cilia of a cirral sheet were observed in *D. polymorpha* nor have any been reported in previous descriptions of the cirri of bivalve gills (Owen, 1974; Owen and McCrae, 1976; Gibbons, 1961).

The number of cilia forming a cirrus in *D. polymorpha* varied but was between 38 and 42. Each cilium exits the apical side of the cell such that it becomes a component of one of the paired sheets. For most of its length, the ultrastructure of each cilium was unexceptional (Sleigh, 1962): *i.e.*, a regular 9 + 2 axoneme (Fig. 5a) containing all of the normal structures, including dynein arms, radial spokes, and nexin. Midway up the length of a cirrus, each cilium in the sheet showed axonemal substructures in register (similar alignment) with its neighbors, indicating that the patterns of beating of all the cilia in a cirrus are coordinated: *i.e.*, each cirral sheet beats as a unit. This was confirmed by 'real-time' confocal observations.

Two marked differences between cirral cilia and "typical cilia" are important to cirral function (Figs. 5, 6): a distinct "hinge" region at the base of each cilium (Fig. 5a, b), and a long free apical tip region in which the number of axonemal microtubules is reduced to a small number of individual microtubules (Fig. 5c).

Each cilium in a sheet has a free tip, and the length of each cilium varies along the face of the cirrus with the shortest cilia located frontally (Figs. 4, 6). In contrast, the lengths of the free tips of the cilia are relatively constant across the entire face of the cirrus (Fig. 6a), although the diameter is progressively reduced (Fig. 5c). The initial reduction of the axoneme as it forms the free tip is abrupt (Fig. 6a, b). A shoulder region, formed by the successive reduction of the outer microtubular doublets to singlets and a shift to a more eccentric position of the central microtubular doublet, forms a distinct bulge on one side of each cilium. The shoulder region is roughly 0.9 μm in length, with the bulge forming about 0.5 μm of that length. This region has been described previously for the cirri of *Mytilus* by Owen (1974). In *Mytilus*, the region was described as having a stiffening rod associated with the free tip, and doublet #1 was indicated as being associated with the stiffening rod. In *Dreissena*, an electron-dense material connects the central microtubule doublet to outer doublet #1, but the material is not as extensive as the stiffening rod described by Owen (1974) for *Mytilus*. At the end of this abrupt shoulder region, only a single outer microtubular doublet remains, and the realignment of the central doublet to a more eccentric position almost perpendicular to the remaining outer doublet is evident. Further toward the free tip, the singlet microtubules begin decreasing in number (Fig. 5c). While the pair of microtubular doublets in each cirral cilium described above define the extended organization out to the tip, the number of other microtubules in the axoneme continued to decrease along the length of the free tips (Fig. 5c).

The presence of the shoulder region causes the fluorescent "beading" at the end of the fused cirral body observed in confocal images (Fig. 4). The nonfluorescent band between the free tips of the cirral cilia and the fused

cirral base in static laser confocal images made the cirrus in such images appear discontinuous (Fig. 4). This apparent discontinuity occurs because the conformation leaves this region specifically out of the plane necessary for fluorescent visualization and causes a lack of reflection in the proper focal plane for confocal microscopy in a single Z plane. At the shoulder, the free tips of the dorsal cirral sheet form an acute angle oriented dorsally, and the ventral cirral tips are bent ventrally.

The "hinge" region of each cirrus is located at its base. Its position is just under the microvillar layer of the latero-frontal cell, but above the cell surface, at a height about 0.2 μm or about 1/3 the length of the microvilli (0.7–0.8 μm). The hinge region has multiple morphological components and is about 200 nm long. The hinge region is narrower than the rest of the axoneme proper (Fig. 5b). The central doublet of the axoneme inserts onto an electron-dense basal plate of the hinge region distal to the cell surface (Figs. 5a in cross section; 5b in longitudinal section). The outer doublets are surrounded by an electron-dense material along the length of the hinge region (Fig. 5a, b). In longitudinal section, there are two electron-dense "plates" at the base of the hinge that span the axonemal region (Fig. 5b). The distal plate is thicker than the basal one and is continuous with the peripheral electron-dense material described above. These "plates" of electron-dense material are also observed in cross section (Fig. 5a). The hinge region is distinct from the junction of the basal body and the cilium, in that the outer microtubules are in the form of doublets both above and below the hinge plates (Wheatley, 1982).

Cycle of cirral movement and particle capture

A laser confocal microscope collecting images at rates from 30 to 240 frames/s allowed observation of the cirral motion in real time. When the latero-frontal cirri are extended, they project perpendicular to the filament into the interfilament space at the level of the latero-frontal cell from which they originate (Fig. 7a). The two sheets of fused cilia composing a cirrus are arranged in a dorsal-ventral orientation. The tips of the cirral cilia (CC) located on the dorsal sheet splayed out dorsally; the ventral CC tips, ventrally (Fig. 7b). The departure of the tips from the plane of the cirral body impart a feather-like appearance to the cirral pair (Fig. 7a, c). The free tips of the CC are sufficiently long that the tips of adjacent cirri form a meshwork or "net," with the spaces between the tips having submicron dimensions (Figs. 7a, c). This cirral net has been described for several other species previously (*e.g.*, Owen and McCrae, 1976; Ribelin and Collier, 1977).

The cirrus moves to a flexed position by combining first a pivoting motion at the hinge followed by a coordi-

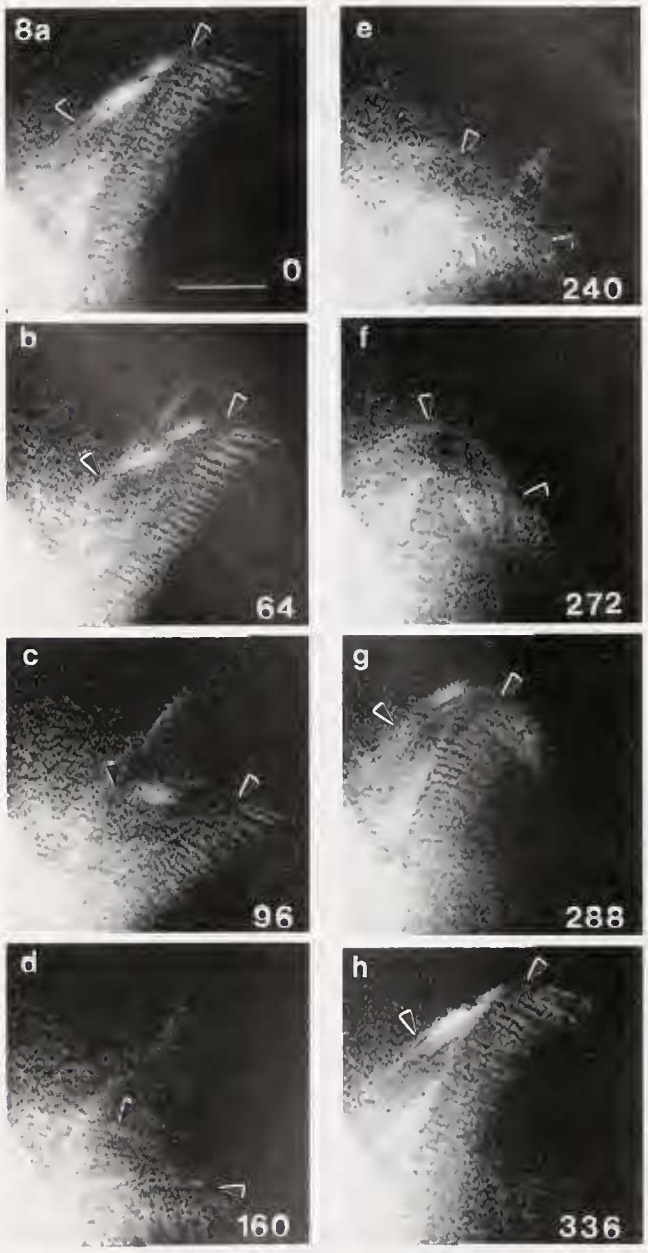

Figure 8. A series of laser confocal video images (120 frames/s) of a cirrus from the gill of a live *Dreissena polymorpha*. The focal plane is perpendicular to the dorsal-ventral axis of the filament and allows observation of one cirral plate and its free ciliary tips. These static images show one beat cycle of a cirrus. The images also contain the beat of a neighboring cirrus in the background. The first image in this series is designated time 0. Each additional image in the series shows the time in ms (lower right hand corner) elapsed from the first image. The power stroke took about 200 ms, while the recovery stroke took only 136 ms. Movement of the hinge region and the waveform beat of the cirrus is evident. The two arrowheads in each micrograph denote the mid-region of the cirral plate and the junction of the cirral plate with its free ciliary tips. Bar = 5 μm.

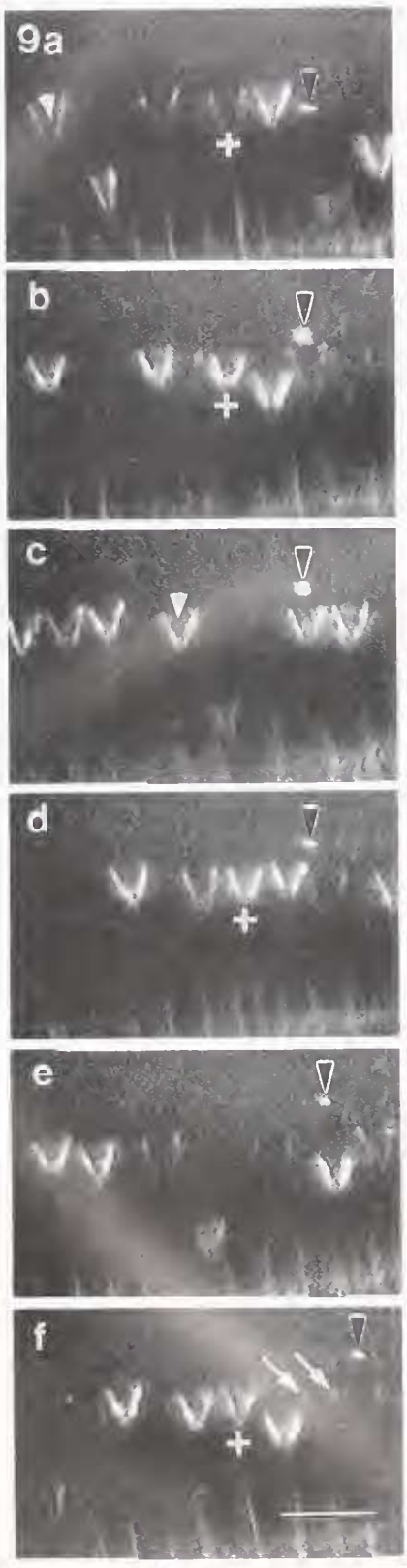

Figure 9. A series of laser confocal video images (30 frames/s) of living *Dreissena polymorpha* gill. The frontal surface of the filament is located at the top of each image. Individual cilia on this surface are out of the plane of focus. The cirri on one side of the filament are visible. The beat of these cirri resulted in a flexion into the plane of focus and over the frontal surface and an extension back and slightly to the left out of the focal plane into the interfilament space. The free ciliary tips of each cirrus form a bright V (white arrowhead; see Fig. 7 a, b for comparison) as the cirrus is flexed over the front of the filament. The width of the open face of these V's is about 2 μm. The dark space below the bright V's is the interfilament space, and the extended cirri drop down and out of the visible plane. The fluorescent linear structures at the base of each image are the fused plates of cirri associated with the adjacent filament located below the bottom of each image. The fluorescent dot (black arrowhead) on each image is a 0.75 μm fluorescent bead, one of many that were added to the preparation and allowed to move across the gill. In (a) the particle (black arrowhead) was free and moving below the front of the filament. In (b) a cirrus was flexing into the plane pushing the particle (black arrowhead) up onto the frontal surface of the filament. In (c) the cirrus was fully flexed and the particle was on the filament. In (d) the particle remained on the frontal surface as the cirrus with which it had interacted extended. In (e) the adjacent cirrus nears the particle as it moves into a flexed position. In (f) the cirri near the particle have extended out of the focal plane. In both (e) and (f) the particle moved to the right along the frontal surface. In this time series the velocity of the particle along the frontal surface of the filament was 25 μm/s. This time series also indicates the beat pattern of a group of eight to nine adjacent cirri. For reference, a single cirrus is identified (+) throughout the time sequence on the images where it is visible. The beat pattern was such that some cirri were flexing while others were extending. All of these cirri were beating rapidly (averaging about 15 beats/s for an individual cirrus). The movement of the particle along the frontal surface was due to the beat of the frontal cilia, which were not, however, in the focal plane of this set of images. Finally, a few of these images suggest the presence of the pro-lateral frontal cilia (arrows, Fig. 9f). Bar = 5 μm.

nated curvature of its component cilia (Fig. 8). This moves the cirrus to an arched frontal position extending over the frontal surface of the filament (Fig. 7a). In some cases, flexed cirri from opposite sides of the filament extended far enough so that, together, they covered about 50% of the frontal surface of the filament. In this flexed forward projection, the cirri appeared to form open "V's" as viewed frontally with scanning electron microscopy (Fig. 7a, b).

A time series captured by laser confocal microscopy at 120 frames/s is shown in Figure 8. The entire cycle for this particular cirral beat was 336 ms. The images were chosen to represent the beat cycle, and the time elapsed from the first image is indicated on each of the subsequent images. The power stroke took about 200 ms (Fig. 8a–d) while the recovering stroke took another 136 ms (Fig. 8e–h). The movement on the basal hinge described above and the gradual curvature of the ciliary bodies during the flexing movement of the cirrus can be seen. The more rapid whip of the cirral cilia back toward the extended position, as well as the movement on the basal hinge, can be observed. Cirri often move faster than the one presented in this time series, but the beat process and the ratio of time spent in each component of the cycle

remain similar for faster-beating cirri. The beat pattern observed here is similar to that described by Dral (1967, 1966) for the cirri of *M. edulis*.

Figure 9 is a set of static images from a time series captured by confocal microscopy; it shows a representative interaction between a 0.75 μm fluorescent particle and a cirrus. The interval between each frame is 33 ms, except that between (e) and (f) which is 66 ms. The identified particle was moving freely before the initial frame (Fig. 9a): then it interacted with a cirrus and was pushed toward the frontal surface of a filament (Fig. 9b, c). Once the particle was pushed up onto the front of the filament (Fig. 9d), it was quickly moved along the frontal surface. In the 100 ms that elapsed between Figures 9d and f, the particle moved 2.5 μm along the frontal surface. This time series is typical of our observations and indicates the capture and movement of such particles to the frontal surface by an individual cirrus. In cases where the first sweep of a cirrus did not successfully move the particle onto the frontal surface, the particle often interacted with the same or several other cirri until it was pushed far enough onto the frontal surface that it began moving along that surface. Similarly, particles that interact with an extended cirrus were pushed forward with the flexion of the cirrus to the same position as seen in the example (Fig. 9).

The most lateral of the frontal cilia (pro-lateral-frontal cilia) were also observed to move into the open "V" face of the cirrus with a beating motion that would appear to aid in the transfer of particles from the cirral face onto the frontal cilia (Fig. 9). The branches of the "V" are composed of the aggregated ventral and dorsal CC tips, and the base of the "V" is composed of the dorsal and ventral faces of the fused sheets. The open face of the "V" is about 2 μm across.

Discussion

The results of this study clearly indicate that cirral structure, position, and motion in *Dreissena polymorpha* create a "capturing net," and that the net is active in particle capture. This does not exclude a role for the branchial water currents created by cirral movement (Jørgensen, 1981; Nielsen *et al.*, 1993); it simply indicates that cirral "net" filtration is indeed an important component of small particle capture by the gill in *D. polymorpha*. Atkins (1937, 1938) described with remarkable accuracy the existence and structure of laterofrontal cirri on bivalve gills from many different lamellibranch families. She recognized that these cirri are formed from multiple fused cilia. Cirral structure has subsequently been described for a number of different species using ultrastructural techniques. The species most often studied to date have been *Mytilus edulis* and *Crassostrea virginica*. *C. virginica* has a plicate, heterorhabdic gill. It has rather small cirri, with each cirral plate containing less than 20 cilia, and with cirral cilia slightly more numerous on ordinary filaments than those on principal filaments (Ribelin and Collier, 1977). The cirri near the marginal groove of these filaments are very small, composed of only 3–4 cilia per plate (Ribelin and Collier, 1977). In contrast, *Mytilus edulis* has a filibranch gill. The cirri of *M. edulis* have been extensively described and are rather complex, containing approximately 30 cilia per plate (Gray, 1922; Moore, 1971; Owen, 1974; Owen and McCrae, 1976; Jones *et al.*, 1990). Both of these species demonstrate cirral nets formed by free ciliary tips in ultrastructural studies (Moore, 1971; Owen, 1974; Owen and McCrae, 1976; Ribelin and Collier, 1977).

Atkins (1938) subdivided the bivalves into the Macrociliobranchia and the Microciliobranchia based on the presence or absence of cirri, respectively. Interestingly, because of the relatively small size of cirri in the Ostreidae, Atkins included this family in her Microciliobranchia. Owen and McCrae (1976) in their discussion also note that the size and structure of the cirri on bivalve gills vary extensively with species and gill type. Owen and McCrae (1976) further relate differing particle capture mechanisms to the two groups (placing Atkins's outlier the Ostreidae into the Macrociliobranchia), suggesting that current flow and mucus are the dominant mode for particle collection in the Microciliobranchia, and that actual particle capture occurs in the Macrociliobranchia. The complexity of cirral structure in *D. polymorpha* is more similar to those found in *M. edulis* than those in *C. virginica*.

Dreissena polymorpha has high particle filtration rates compared to other freshwater mussels (Jørgensen *et al.*, 1984; Kryger and Riisgård, 1988; Bunt *et al.*, 1993; Silverman *et al.*, 1995), especially for particles smaller than 2 μm. The location, orientation, and dynamics of the latero-frontal cirri on the gill of *D. polymorpha* suggest that they are responsible for particle capture. Cirral motion and interaction with fluorescent beads, as observed by confocal microscopy, confirm cirral involvement in the particle capture process. The "capture net" that forms across the interfilament space by cirri in the extended position is readily apparent with SEM. The size of the spaces formed by the net are in the right order of magnitude (0.2–0.7 μm) to allow the capture of particles attributed to *D. polymorpha* (Morton, 1971). Whether capture is by actual trapping within the "net" or whether the net is behaving more like a "solid" capture sheet remains to be determined (Purcell, 1977).

The hinge mechanism on the cirrus allows it to assume, alternately, an extended or flexed orientation rel-

ative to the position of the latero-frontal cells on which it originates. The motion of the cirrus suits both an initial capture event and the delivery of captured particles onto the frontal cilia for further processing. The cirral hinge region has been described previously as the ciliary neck region of unknown function, but showing specialized membrane structure by freeze-fracture examination (Good *et al.*, 1990). The static images of the SEM indicate the potential, and the dynamic images of real-time laser confocal microscopy demonstrate the actual process of cirral movement as it occurs.

We saw no evidence, either in live isolated gills or SEM samples, that mucus contributes in a major way to this component of particle capture. There are no obvious mucus cells located on the gill filament except at the base of the filament near the external ostia. No mucus production was evident in the vicinity of the cirri. There does not appear to be a major requirement for mucus participation in the capture of small particles in *D. polymorpha,* at least in the isolated gills observed in this study. Mucus cells are present below the level of the lateral ciliated cells and are prominent at the entry of the water canal into the central water channel and along the water channel epithelium. Mucocytes are also prominent at the dorsal margin leading to the suprabranchial chamber. Mucus clearly plays a role in particle transport (Beninger *et al.*, 1992; Ward *et al.*, 1993) and food processing in a wide variety of bivalves. In addition, our preliminary studies on excised unionid gills indicate that mucus is conspicuously associated with cirri in several species (unpubl. obs).

Although we have examined only one species to date in detail, so extensive speculation is not warranted, the mechanism described here for capture of small particles (≤ 2 μm) may explain the feeding data presented in several studies. These studies have shown that species with complex cirri are more efficient in removing small particles than those species with less complex cirri, or with no cirri at all (Owen and McCrae, 1976; Møhlenberg and Riisgård, 1978; McHenery and Birbeck, 1985; Riisgård, 1988).

The mechanism of particle capture has been debated, yet there may not be a single mechanism, but rather a suite of mechanisms contributing to particle capture by bivalves with widely differing demibranch structures (Ward *et al.*, 1993). Jørgensen (1981, 1982, 1989) indicates that these cirri along with the other ciliary components of the filament create a set of three-dimensional currents that act in concert to entrain particles in the water column. The hypothesis is based on various current measurements and interpretation of other studies indicating that the cirral mesh should trap all particles above a certain size. This hypothesis has not been confirmed by experimental observation (Vahl, 1972; Møhlenberg and Riisgård, 1978). Our results indicate that cirri comprise a moving, dynamic mesh that will not be capable of capturing every particle, particularly at the lower limits of the mesh size. When a single cirrus is bent frontally it would not be in position to exert a sieve effect. This suggests that the trapping efficiency of the cirri cannot be 100%, particularly at particle sizes approaching the lower limits of their capturing ability. Our interpretation agrees with the observations and calculations of Silvester and Sleigh (1984; Silvester, 1988). Our observations coincide with the experimental data of Owen and McCrae (1976) indicating that the mesh size of the cirral "net" in a few selected species of marine bivalves is roughly correlated with the size of particles filtered by a particular species. Silvester and Sleigh (1984) provided physical calculations indicating that the size range and quantity of particles actually trapped by gills under various experimental conditions are consistent with the cirral mesh size in *Mytilus.* Comparisons of several marine species by Riisgård and colleagues suggest that larger, more complex cirri may be associated with the ability to trap particles on the order of 2 μm (Møhlenberg and Riisgård, 1978; Riisgård, 1988). McHenery and Birbeck (1985) indicate that marine species with complex cirri (*Mytilus edulis*) are better able to clear *Escherichia coli* than bivalves with less complex cirri (*Ostrea edulis*), whereas a species with no latero-frontal cirri (*Chlamys opercularis*) is unable to capture bacteria. Silverman *et al.* (1995) have also shown that in three species of freshwater bivalves, the clearance of *E. coli* is correlated with the structural complexity of the gill cirri.

By describing the "capture net" of *D. polymorpha* with mesh spacing between 0.2 and 0.7 μm, we raise the issue of fluid and particle movement that may be more affected by viscosity than inertial forces (*i.e.*, low Reynolds number effects). In several other species (echinoderm larvae, rotifers, copepods), studies on particle capture by structures with small net spacings have indicated that such structures act more like solid paddles moving fluid than as actual sieves capturing particles (Strathmann, 1971; Strathmann *et al.*, 1972; Koehl and Strickler, 1981; Strickler, 1982). The size range and structure of the cirri clearly suggest low Reynolds number fluid mechanics (Rubenstein and Koehl, 1977). Under steady state conditions, mathematical modeling and experimental testing of the capture mechanism of nets or sieves of this type indicate that they would act more like paddles than nets (Rubenstein and Koehl, 1977; Shimeta and Jumars, 1991). However, for the gill of *Mytilus edulis,* Nielsen *et al.* (1993) suggest, from partial filament model systems, that the interfilamental area of the gill is not strictly in a steady flow state due to the velocity differences in this region caused by the activity of the ciliary pumps. Shimeta and Jumars (1991) suggest that under nonsteady state conditions particle movements and

interaction with filter elements may vary considerably from that predicted by steady flow modeling.

Confocal microscopy confirms that cirri trap or deliver 0.75–1 μm fluorescent beads to the frontal surface of the gill. The movement of a 0.75 μm particle by a cirrus onto the frontal surface of the filament is shown in the time-lapse images captured using laser confocal microscopy (Fig. 9). These images by themselves do little to confirm whether the delivery mechanism is strictly hydromechanical or whether some particle contact with the cirri occurs. Given the unsteady flow environment, only exacting measurements of fluid velocities at the cirral sites themselves, identification of particle trajectories, and measurement of shear and drag forces will allow the exact mechanism of cirral interaction with particles to be determined. What does appear to be clear from the data presented in Nielsen *et al.* (1993) for *Mytilus* and for our confocal observations of *Dreissena* is that a close interaction between gill cirri and particles results in particle capture.

We have observed here that cirri can capture particles and that isolated capture events can be observed by confocal microscopy. The observations have only been made in a single species, but they demonstrate the potential role that cirri can play in particle capture by bivalves. What is not demonstrated by these observations is whether particle capture by cirri is the predominant mechanism of particle capture or whether other capture mechanisms provide for more particle capture by a gill than the cirral mechanism. The answer to this question is likely to vary with species, and particularly to variations in cirral structure and overall gill structure. For a given species, the relative roles of hydrodynamics and the mechanics of inertia and viscosity in suspension feeding events will therefore depend on structure, assortment, and location of the locomotory (ciliary) elements, the mesh size of cirral nets, the diameters of the pores, and the sites and amount of mucus release. The assumption that modest differences in cirral structure are not important to function because they are all acting similarly as paddles, fails to take into account that overall gill function in each bivalve is determined by a suite of variable characters whose functional interrelationships are crucial to the mechanisms of particle capture.

Observation of the intact, functioning gill within the pallial cavity is clearly the optimal way to account for all forces and movements that occur in that complex fluid environment. Endoscopy has provided the data for the particle transport process (Ward *et al.*, 1993), but until a resolution of about 0.5 μm is achieved, this method alone will not provide the information necessary to address the issue of small particle capture by the gills of bivalves. Laser confocal microscopy does allow real-time observation of interactions between cirri and small particles, but requires excision of the gill. Continued comparative studies of gill musculature, motile organelle dynamics, and the architecture of water canals and channels is required for a more complete understanding of particle capture during water movement through and across the gill.

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